The University of Chicago

THE THYMUS GLAND IN RELATION TO SEX HORMONES AND REPRODUCTIVE PROCESSES IN THE ALBINO RAT

A CONDENSATION OF A DISSERTATION SUBMITTED TO THE FACULTY OF THE DIVISION OF THE BIOLOGICAL SCIENCES IN CANDIDACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOOLOGY

1940

By

JAMES C. PLAGGE

Private Edition, Distributed by
THE UNIVERSITY OF CHICAGO LIBRARIES
CHICAGO, ILLINOIS

Reprinted from
JOURNAL OF MORPHOLOGY, Vol. 68, No. 3, May 1, 1941

The University of Chicago

THE THYMUS GLAND IN RELATION TO SEX HORMONES AND REPRODUCTIVE PROCESSES IN THE ALBINO RAT

A CONDENSATION OF A DISSERTATION
SUBMITTED TO THE FACULTY OF THE
DIVISION OF THE BIOLOGICAL SCIENCES
IN CANDIDACY FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOOLOGY
1940

By

JAMES C. PLAGGE

Private Edition, Distributed by
THE UNIVERSITY OF CHICAGO LIBRARIES
CHICAGO, ILLINOIS

Reprinted from
JOURNAL OF MORPHOLOGY, Vol. 68, No. 3. May 1, 1941

R. UNIV.
BIBLIOTHEEK
LEIDEN

Reprinted from the JOURNAL OF MORPHOLOGY
Vol. 68, No. 3, May, 1941

THE THYMUS GLAND IN RELATION TO SEX HORMONES AND REPRODUCTIVE PROCESSES IN THE ALBINO RAT [1]

JAMES C. PLAGGE
Hull Zoological Laboratory, The University of Chicago, Chicago, Illinois

EIGHT FIGURES

INTRODUCTION

Investigations carried on in this laboratory for many years on the biology of sex, including various reproductive phenomena, have involved a study of the effects of various endocrine glands upon the reproductive system as well as the interrelationships existing between this system and certain of the organs of internal secretion (Moore and Price, '32). Since the claim that the thymus gland is an organ of internal secretion has been both affirmed and denied, and since some references indicate a connection between the thymus gland and the reproductive system, it was considered advisable to attempt a comprehensive study of the possible interrelationships that may exist between the thymus, gonad activity and reproductive processes in general.

This study resolved itself into two main problems: (1) Does the reproductive system of the albino rat have any effect on the activity or condition of the thymus? and (2) Does removal of the thymus gland, as has been alleged, influence the development of reproductive functions? If such a reciprocal relationship between the thymus and the gonads

[1] This investigation has been aided by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of The University of Chicago.

could be established, it would be evidence for the claim that the thymus is an endocrine gland.[2]

MATERIALS AND METHODS

Approximately 1400 albino rats from the stock colony of this laboratory were studied during a 4-year period. The normal growth rate and involution period of the thymus were determined in 496 male and female animals. The remaining rats were castrated, injected with sex or gonadotropic hormones or thymectomized. In all experiments a litter was used as the unit.

Experimental animals and their littermate controls were kept together under the same conditions. Prior to autopsy each animal was weighed after which it was killed by decapitation. The thymus, gonads and accessory reproductive organs were removed, weighed to 0.1 of a milligram and fixed for histological purposes. A more complete account of this study may be found elsewhere (Plagge, '40 b).

NORMAL GROWTH AND INVOLUTION OF THE THYMUS

Although it has long been known that the thymus begins to degenerate early in life, undergoing a so-called "age involution" at a much earlier date than the other organs of the body, it was necessary to have exact data on this process in normal animals of this colony in order to make adequate comparisons with treated ones. Therefore, 284 normal male and 212 normal female albino rats were killed at frequent intervals between the ages of 1 day and 600 days. The age

[2] This problem was suggested to me by Professor Carl R. Moore. His constant cooperation in offering advice and criticism has greatly aided the progress of the study. I also appreciate the kindness of Professor Sewall Wright for his suggestions in the analysis of data. It is a pleasure to acknowledge those who have supplied hormone preparations: Dr. Gregory Stragnell, of the Schering Corporation, for testosterone and its compounds and for progynon-B; Professor L. Ruzicka, C. C. Haskell and the Ciba Company, for androsterone; Dr. Oliver Kamm, of Parke Davis and Company, for theelin and antuitrin-S; Dr. J. A. Morrell, of Squibb and Sons, for prospermine; and Dr. Donald H. Wonder, of the Cutter Laboratories, for gonadin.

of maximum growth and of beginning of involution of the thymus were then compared with the age at which sexual maturity is attained. The animals of any given litter were killed at varying ages to avoid the concentration of possible litter peculiarities at any particular age.

The growth phases of the thymus in male rats are shown graphically in figure 1. The points represent the distribution

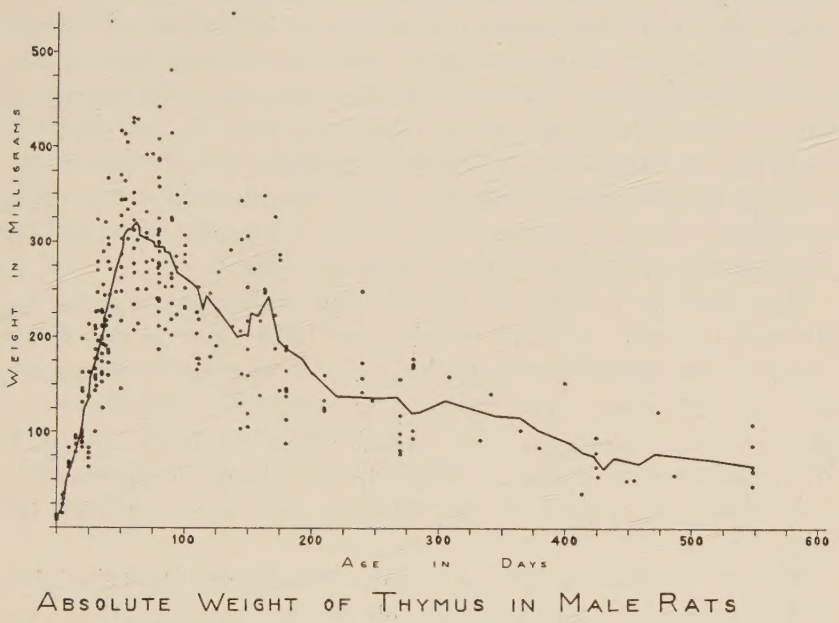


Figure 1

of weights of the thymus for individual animals. The line indicates the moving average of weighted means for both weight and age values in groups of five.

The absolute weight of the thymus in male rats increases rapidly to a maximum of about 300 mg. at approximately 60 days. It then decreases rather abruptly with a slight leveling off between 200 and 300 days. Thereafter, the thymus continues to become smaller at a slower rate, never weighing more than one-third its maximum.

The growth and involution of the thymus in female rats are not essentially different from those of the males. The maximum weight of about 275 mg. is also reached at approximately 60 days. Definite signs of involution occur at about 125 days, apparently a little later than in males. These data are not essentially different from those given by Donaldson ('24) for albino rats from birth to 400 days of age.

The maximum size of the thymus in the rat is reached somewhat later than the beginning of sexual maturity. Browman ('37), in observing dates of first litters for rats of this colony, noted that 74 days was the earliest age at delivery, from brother-sister matings and that 65 days was the earliest age at delivery, from father-daughter matings. Subtracting the 21 days of pregnancy, females were sexually mature as early as 45 days of age and males at 55 days, periods at which the thymus was still increasing in absolute weight.

The relative weight of the thymus (absolute weight expressed in terms of body weight) for both sexes is relatively high at birth and is increasing rapidly. It reaches its maximum at about 25 days. The relative weight then commences to decrease rapidly during the period when the body weight is increasing most rapidly. From 200 to 600 days the relative and absolute weights of the thymus decrease at about the same rate.

Before involution of the thymus has begun its two lobes are in intimate relation with each other. As involution progresses this close contact is gradually lost. When the rat is 400 to 600 days old the two lobes have separated and are completely surrounded by adipose tissue. The reduction in size results in diminished thickness of the lobes without change in their length.

Histologically, the cortical and medullary components of the thymus retain a clear-cut differentiation during the time it is increasing in absolute weight. Histological evidences of involution are not usually apparent until some time after the thymus begins to show decrease in absolute weight. Although the time of onset of these changes varies considerably,

most rats studied began to show definite indications of age involution by the time they were 200 days old, at which time the thymus of both males and females weighed approximately one-half of its maximum. The changes, which gradually become more prominent after 200 days, consist of a decrease in number of thymocytes in the cortex and a fading out of the boundary between cortex and medulla. In cases of extreme involution, as seen in the oldest animals studied, the medullary tissue also shows a thinning out of thymocytes and to some extent replacement by fatty tissue (compare figs. 4 and 7). The medulla frequently extends to the periphery of the gland in these older animals; the cortex is represented by an occasional darkly staining nodule. The epithelial reticular cells, into whose meshes the thymocytes are packed, are shrunken and replaced by fat and connective tissue. Hassall's bodies, never numerous and seldom distinct in the rat thymus, are still found after extensive involution.

EFFECTS OF REPRODUCTIVE SYSTEM ON THYMUS

Effects of castration

Castrations were performed on approximately 75 male and 75 female rats between the ages of 1 day and 546 days. Fifty-five of each sex were gonadectomized between the ages of 1 and 80 days, before the thymus showed marked involution and the remaining rats were castrated between the ages of 206 and 546 days, at which time the thymus weighed less than 50% of its maximum. Each litter group was checked against an approximately equal number of unoperated littermate controls. Thirty-one litters were operated in the 1-to-80-day age group, and eighteen litters in the advanced age group.

Castration before involution of the thymus. Since it appeared from a preliminary study of the normal thymus that marked involution did not occur until after 80 days, the pre-involution castrate series was designed to test the effects on the thymus of gonadectomy from birth to 80 days. Animals

were segregated into three major groups according to their age at the time of gonadectomy and of autopsy: (1) castration at 1, 30 or 50 days and autopsy at 80 days; (2) castration at 20 days and autopsy at 91, 182, 273 and 547 days; and (3) castration at 80 days and autopsy at 150, 180, 210 and 240 days.

The data for the male rats of this castration series are summarized in a bar graph (fig. 2) in which the thymus

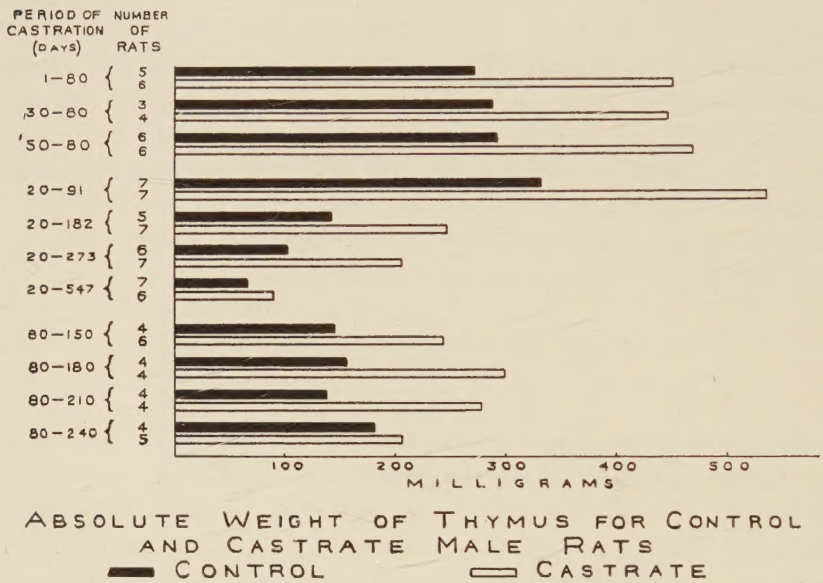


Figure 2

weights of controls are represented by solid bars and of castrates by open bars.

The top three pairs of bars in figure 2 indicate that increases in weight of the thymus of male rats killed at 80 days of age are about the same for animals castrated at 1, 30 or 50 days.

The results for the group of rats castrated at 20 days of age are shown by the next four pairs of bars. The drop in normal weight of the thymus from 91 to 547 days of age is shown by the successively shorter black bars. The corresponding white bars indicate that, while there is an appreciable

increase in the weight of the thymus following castration at 20 days, the involution of this gland proceeds much the same as in the unoperated animal. The time of beginning of the involution of the thymus apparently is not dependent upon the sex organs or sex maturity. From somewhat similar data this idea was first suggested by Chiodi ('38 a). Castration does not seem to delay involution as has frequently been reported (Hatai, '15; Marine, Manley and Baumann, '24; Korenchevsky and Dennison, '34). The longest period of castration, 20 to 547 days, resulted in only slight increase in the weight of the thymus in males and an actual decrease in the weight of the thymus in females. Several of these animals had lung infections which may have produced accidental involution of the thymus, obliterating the hyperplastic effects of castration. A correlation was noted in normal 500- to 600-day-old rats between the presence of such infections and thymus atrophy. The same correlation has been noted by Hatai ('14).

The lower four pairs of bars in figure 2 indicate that castration at about the time of puberty also results in increasing the weight of the thymus when the animals are killed at 150, 180, 210 and 240 days of age.

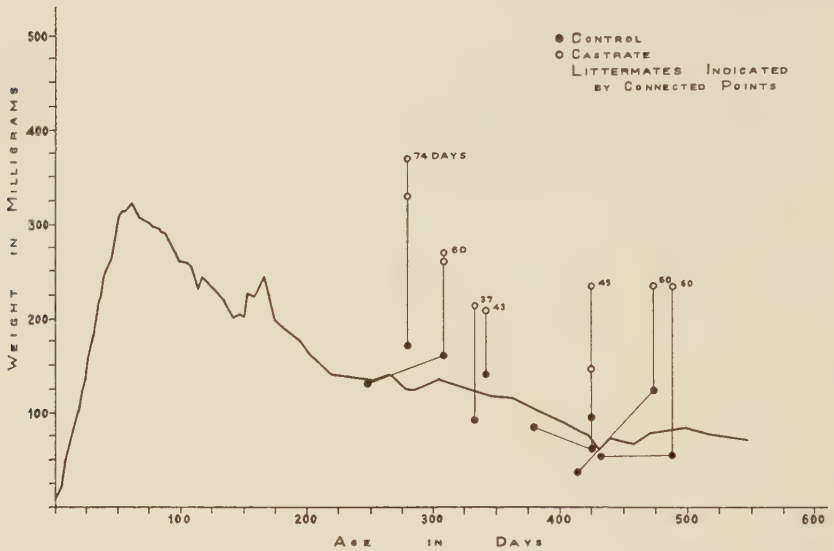
The female rats which have been castrated for the same periods as the males show similar increases in gross weights of the thymus. The relative weights of the thymuses of both males and females are also proportionally increased.

In each of three litters of rats one individual was unilaterally castrated. The thymus glands of these three rats did not increase in size more than those of the unoperated controls, whereas the bilaterally castrated members showed pronounced hyperplasia of the thymus.

Castration after involution of the thymus. The above data indicate that gonadectomy at prepuberal or puberal ages leads to an increase in the weight of the thymus irrespective of the age of killing. The next point of interest is a consideration of the effect of gonadectomy in older rats, after the thymus has decreased to a small fraction of its greatest

weight. An abstract of these findings has been published (Plagge, '39). The data will be considered here in somewhat more detail.

Sixty-two male and female rats (including test and control animals) were castrated between the ages of 206 and 546 days of age. Periods of castration ranging from 10 to 74 days resulted in increases in gross weights of the thymus ranging



Effect of Gonadectomy on Absolute Weight of the Involuted Thymus in Male Rats

Figure 3

from 33% to 502% and in similar increases for relative weights.

Seven of the twelve male littermate groups that were castrated at advanced ages are presented graphically in figure 3. The experimental data are superimposed on the curve of normal growth and involution of the thymus. Some controls were killed on the day of operation and others on the day of autopsy of castrates. The duration of the period of castration is indicated by the numbers opposite open circles.

Figure 3 indicates that there is a marked increase in the weight of the involuted thymus of male rats during periods of from 37 to 74 days after castration. Equivalent treatment of females resulted in similar increases in weights of their thymuses when the glands were taken at ages ranging from 360 to 606 days. In many animals the involuted thymus of 50 to 100 mg. returned nearly to its maximum weight of 250 to 300 mg. In the thirty-three animals that were castrated the response of the thymus bore no clear-cut correlation to the length of the period of castration, the age of the rat or the extent of thymic involution. For example, in some cases gonadectomy in 500-day-old rats, in which the thymus normally weighs only 50 mg., resulted in the same hyperplasia observed in the less involuted glands of 250-day-old rats.

Not only does the involuted thymus in a castrated rat return to a weight approximating that of a much younger animal but also there is a restoration of its former gross and histological appearance. The thymus of a 400- to 600-day-old rat consists of two separate and uniformly thin, long, finger-like strands of soft tissue, only partially filling the anterior thoracic cavity. The thymus glands after relatively short periods of castration closely resemble the squat, pyramidal shape of the puberal animal in which the base of the pyramid is molded to the pericardium and its apex extends toward the head. Again the two lobes are closely connected by connective tissue.

Distinct histological changes leading back toward the pre-involuted type of gland are correlated with the increase in weight and change in form. The cortex which had almost disappeared is reestablished and may again be clearly distinguished from the medulla. Figure 7 is a cross section of the thymus from a normal male at the age of 425 days. This represents typical age involution. Cortical areas are almost gone, and degeneration is well advanced. The thymus of a littermate brother is shown at the same magnification in figure 6, but this animal was castrated 45 days before it was killed. This is an instance of advanced hyperplasia of the

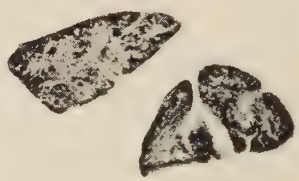


Fig. 4 Cross section of untreated rat thymus at 38 days of age (× 12.3).

Fig. 5 Thymus from littermate control of animal in figure 4, showing effect of 16 daily injections of 100 rat units of prospermine (× 12.3).

Fig. 6 Thymus from 425-day-old male rat, castrated for 45 days (× 12.3).

Fig. 7 Thymus from an unoperated littermate brother of animal in figure 6 (× 12.3).

involuted thymus within a period of 45 days following castration. The gland resembles the normal 38-day-old tissue (fig. 4). Cortical and medullary regions are present in nearly normal relationships with a distinct boundary between the two. In earlier stages of recovery dark cortical nodules appear first near the center of the gland and then digitate out to the periphery.

In summary, either pre- or postpuberal castration of male or female rats results in definite hyperplasia of the thymus. Although prepuberal castration causes increases in thymus weights of 50% to 100% over those of littermate controls, the time of onset of involution at about 80 days is not altered. The effects of castration upon the thymus of the infantile rat are prolonged several months beyond sexual maturity, but they are no longer apparent in the 547-day-old rat. However, the involuted thymus at this advanced age is capable of responding dramatically to castration by hyperplasia after relatively short time intervals. These changes make it approximate the none-involuted thymus.

Effects of hormone injections

It would appear from the foregoing castration experiments that the increase in weight of the thymus after castration may be due to the removal of sex hormones. It was decided, therefore, to inject sex hormones and gonad-stimulating substances into normal and castrated animals in order to determine their effects on the thymus (table 1).

Representative rats from forty-six litters, comprising 254 animals in all, were injected. In most cases there were at least two untreated controls in each litter.

Sex hormones. Either male or female sex hormones injected into either castrated or unoperated male or female rats caused reductions in weight of the thymus up to 71%. Effects were roughly proportional to the dosage used.

Testosterone propionate was found to be approximately four times as effective in decreasing the weight of the thymus as testosterone in normal animals of equivalent age. This

TABLE 1

Range of effects of hormones upon the thymus

INJECTIONS (DAYS)	SEX	DAILY DOSAGE RANGE IN UNITS	NUMBER OF LITTER-MATES RECEIVING DIFFERENT DOSAGES	PERCENTAGE DECREASE IN THYMUS WEIGHT (CONTROL RATED 1)
Androgenic agents: Testosterone (milligrams)				
15– 30	M	0.25 – 1.0	3	+10*–27
30– 49	M	0.1 – 1.0	3	+20*–11
Testosterone propionate (milligrams)				
5– 19	M	0.05 – 0.2	3	13 –26
5– 24	M	0.05 – 0.2	3	4 –31
10– 29	M	0.05 – 0.2	3	12 –35
30– 49	M	0.05 – 0.3	2	+17*–48
Androsterone (milligrams)				
11– 31	M	0.025– 0.1	3	8 –18
11– 31	M	0.1 – 0.4	4	16 –58
144–163	M	4.0 – 6.0	2	14 –27
Estrogenic agent: Theelin (international units)				
11– 34	M	20.0 –50.0	2	23 –24
16– 35	M	1.0 –50.0	4	5 –25
16– 39	M	20.0 –75.0	3	18 –71
35– 54	M	50.0	3	+34*–25
Gonadotropic agents: Antuitrin-S (rat units)				
10– 29	M	5– 25	3	42 –67
13– 31	M	5– 25	2	48 –68
15– 34	M	25	2	49 –51
18– 37	M	10	2	24 –35
Prospermine (rat units)				
5– 19	M	1– 25	3	23 –42
22– 37	M	25–100	3	71 –82
20– 39	M	1– 25	3	26 –81
40– 59	M	1– 25	3	+21*–61
60– 79	M	1– 25	3	22 –73
80– 99	M	1– 25	3	2 –67
Gonadin (rat units)				
13– 32	M	1– 5	2	56 –71
13– 32	F	1– 10	2	69 –82
76– 95	F	10	2	62 –65
82–101	M	10	2	71 –83
106–125	M	10	1	65

* Animal receiving the lowest dosage showed increase in thymus weight.
Each line represents summary of one litter.
Animals sacrificed 1 day after last injection.

difference in activity is in accordance with the findings of Moore and Price ('38) which demonstrated that testosterone propionate was approximately four times as active as testosterone either in maintaining the normal secretory state of the ventral lobe of the prostate and the seminal vesicle or in restoring long-time castrate tissues to the normal secretory state. In general, the dose of a particular hormone which stimulates the accessory reproductive organs is also effective in diminishing the size of the animal's thymus.

Equivalent weights of androsterone caused decreases in weights of the thymus which were about the same as those resulting from injections of testosterone propionate. A range of 1 to 75 international units of theelin, the female sex hormone, caused reductions in the weight of the thymus ranging from 5% to 71% in male rats in which the thymus had not yet commenced to decrease in size.

The thymus glands of older animals were usually least affected by the hormone. However, in these older animals large doses sometimes caused decreases in the weight of the thymus.

The results of injecting sex hormones into castrated rats were very similar to those obtained in unoperated animals. Since several of the treated litters contained both castrated and unoperated controls, it was possible to distinguish between the effects of castration alone and castration plus hormone treatment within the same litter. In three litters, for example, castration periods of only 20 days caused increases in the size of the thymus of from 5% to 32% above normal; injections of testicular hormones during this period not only counteracted this increase but also caused an actual decrease in the weight of the thymus of from 2% to 45% below normal.

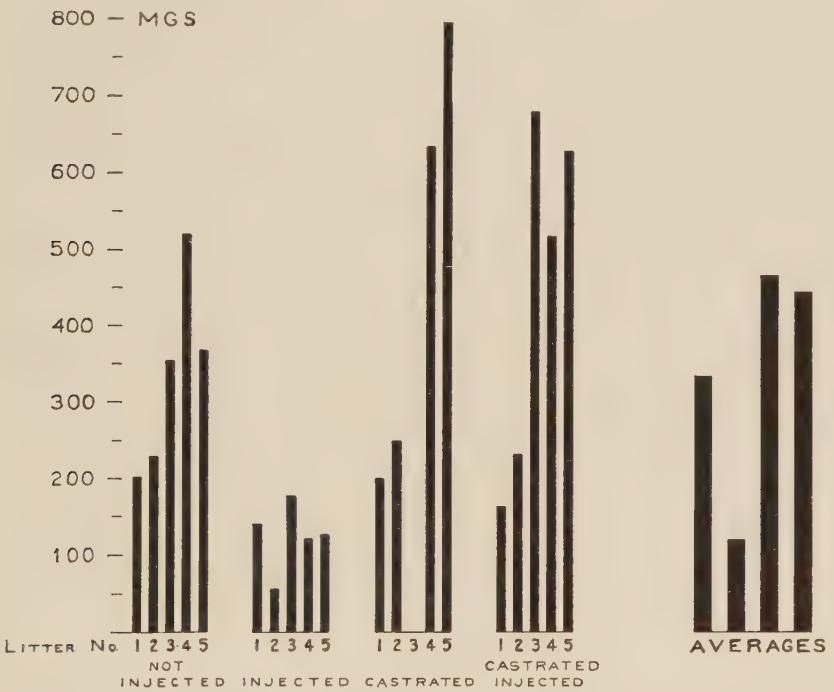
Having demonstrated that the hyperplasia of the thymus resulting from removal of the animal's source of sex hormone can be completely overbalanced by supplying the synthetic form of the hormone, the order of treatment was reversed. A number of 10-day-old animals were injected with theelin

daily for 20 days. At the end of this period representative animals were killed and it was found that the thymus glands had decreased in weight. At the same time the remaining injected animals were castrated and the injections stopped. Fifty days later their thymuses were larger than those of both the normal and the castrate controls. From these results, it is evident that the thymus weight may be first reduced by sex hormones and then increased by castration, the latter process proceeding even to the point of overcompensation.

Gonadotropic hormones. A wide range of dosages of gonad-stimulating hormones (antuitrin-S, prospermine and gonadin) injected at varying ages resulted in decreases in weight of the thymus ranging from 2% to 83% below normal in thirty-five out of thirty-six treated rats. Figure 5 shows a cross section of the thymus of a male rat that was treated with prospermine. The animal received 100 rat units daily from day 22 to day 37 and was sacrificed at 38 days of age with its untreated littermate control whose thymus is shown in figure 4. The magnifications being the same, the reduction in weight from about 300 mg. to about 50 mg. is apparent in the smaller size of the cross section of the thymus of the injected animal. The involution is evidenced also by histological disorganization. Thymocytes have thinned out, leaving relatively more medullary tissue and a less distinct boundary between medulla and cortex.

It would appear from the data just presented that either the male or female sex hormones themselves or the anterior pituitary-like hormones which provoke excessive production of the sex hormones, can cause appreciable involution of the thymus. Do the gonadotropic hormones act directly on the thymus or by stimulating the gonads to secrete more of their own hormones? This question was investigated by injecting castrated animals with gonad-stimulating hormones (antuitrin-S or gonadin). Thirty-five animals in five litters were used. The results are shown graphically in figure 8. It will be seen in the non-castrated animals that the average weight of the thymus of the injected rats is about one-third that

of the uninjected controls. Castrated animals, on the other hand, which were injected with the same quantities of hormone had thymuses which did not decrease in weight. They actually increased to a size almost equal to that of the glands in the castrated uninjected animals. Therefore, the effect of gonadotropic hormones on the thymus is by indirect action through



ABSOLUTE THYMUS WEIGHT OF CASTRATED RATS INJECTED WITH GONADOTROPIC HORMONES

Figure 8

the gonads, causing them to secrete excessive amounts of their hormones.

Combinations of hormones. Nine litters of rats were injected with various combinations of either two or three of the following eight endocrine preparations: testosterone, testosterone propionate, the oxime of testosterone, androsterone, theelin, progynon-B, antuitrin-S and gonadin. Com-

bination effects of hormones in causing thymus involution were roughly equal to the sum of the effects of the hormones when injected separately. For example, 100 international units of theelin injected daily from the thirty-third to fifty-second day of life caused a 35% decrease in the weight of the thymus of two male rats. The thymuses of two littermates that were injected with 25 rat units of antuitrin-S daily were reduced 33% in weight. When the same amounts of these two hormones were injected simultaneously and during the same period into two other littermates, the thymus glands decreased to 73% below normal. Other combinations of hormones gave analogous results.

The results presented to this point show that the thymus of the albino rat (1) increases in size after castration at any age and (2) regresses following injection of sex hormones into normal or castrated animals, or of gonad-stimulating hormones into animals in which the sex glands are intact: the latter procedure is, of course, equivalent to injecting the respective sex hormones that are produced by intact gonads under the influence of gonadotropic agents.

The next phase of this investigation considers the possibility of a reciprocal action between the thymus and gonads. Does removal of the thymus have any effect on the reproductive system?

EFFECTS OF THYMECTOMY

The results of thymectomy at birth in twenty-five out of forty-six rats in eleven litters have been reported (Plagge, '40 a). It was shown that absence of the thymus from birth to 37 days of age neither hastens nor retards spermatogenesis, hormone output or growth of the testes, or general body growth.

The author's previous report did not include observations on approximately 150 additional rats in twenty-two litters that were completely thymectomized at ages ranging from 10 to 16 days. They were killed at ages ranging up to 185 days.

Four litters thymectomized at this later age confirm the conclusion that sperm head formation is not retarded or accelerated by ablation of the thymus. It was found also that body weight was not influenced in the thymectomized members of eight litters when the animals were weighed at intervals of 5 days until they were 80 days old.

Seven of fourteen females in three litters were thymectomized at the age of 11 days. Vaginal canalization of all fourteen rats occurred between the ages of 47 and 60 days. Cornification of the vaginal epithelium occurred regularly at 4- to 5-day intervals in both operated and control animals.

Further observations were made on the weights of the gonads and accessory reproductive organs of both males and females, on sperm motility and on the appearance of hair, eruption of teeth and opening of eyes. No differences between operates and controls were found in any of these developmental criteria. The breeding of thymectomized rats was observed and found not to differ from that of controls. The litters that were cast were similar in all respects. A few of the offspring of thymectomized rats were also thymectomized. They likewise showed no indication of abnormal development. Inbreeding of thymectomized rats was not carried further than the second generation.

The rate of learning of three thymectomized rats was tested with a four-alley multiple choice maze against three littermate sham-operated control rats. The rats were thymectomized at the age of 10 days. First trials in the maze were begun at the age of 29 days. During 22 consecutive days the rats were tested in the maze. Observations were made on time required to run the maze, number of errors made, and activity while in the maze. Although a larger number of animals would be required to determine the validity of the suggestion, there was an indication that thymectomized rats learn a little more slowly than their littermates which have been subjected to an operated control procedure. Aside from this possibility, removal of the thymus in rats produced little or no effect on subsequent learning ability.

In summary, thymectomy of newborn rats neither hastens nor retards spermatogenesis, hormone output, or growth of the testes. Further observations on a limited number of rats thymectomized between the ages of 10 and 16 days indicate that removal of the thymus has no effect on body weight, sexual maturity of males or females, appearance of hair, eruption of teeth, opening of eyes, breeding activity, or ability and rate of learning.

DISCUSSION

The development of the normal thymus gland has been the cause of divergent views for many centuries. By the time it was generally agreed that the human thymus does not reach its maximum weight until about the time of puberty (Hammar, '06), similar discoveries were being made for lower animals from cartilaginous fishes (Hammar, '11) to rabbits (Söderlund and Backman, '09).

The normal development of the thymus of the albino rat was investigated by Jackson ('13) and Hatai ('14). They found that the maximum growth corresponds in general to the time of puberty. Donaldson ('24) grouped the thymus of the rat with the ovaries, testes and epididymides as an organ which showed the most marked rise in growth shortly before puberty.

My observations demonstrate that the thymus of albino rats in this colony reaches its maximum weight in both sexes at about 60 days of age and that involution in the male begins immediately, whereas it appears to be postponed until about 120 days of age in the female. It was pointed out that both males and females may attain full reproductive capacity before the thymus has reached its maximum weight.

While the beginning of involution of the absolute weight of the thymus may be later than the time of attainment of sexual maturity, it was found that the weight of the thymus when related to body weight begins to decrease before the reproductive system is in a full functional state. However, from a consideration of the data of Jackson ('13) a question

might be raised concerning the significance of the early involution of the relative weight of the thymus. A small organ may be increasing in gross weight at a time when its relative weight is decreasing because other larger parts such as the musculature are growing very rapidly. Jackson pointed out that not only the thymus of the rat but also the thyroids, spleen, lungs, heart, adrenals and kidneys reach their greatest relative weight during the first 3 weeks of life. The unique feature of the thymus then would appear to be its abrupt decrease in gross weight when the body weight is still increasing.

The sudden drop in absolute weight of the thymus may be related to gonad development. That the decrease in size of the one structure and the development and functional beginning of the other is just a coincidence has often been suggested. The results obtained by castration and sex hormone administration would not favor this view. Calzolari (1898) was probably the first investigator to observe and publish on the effects of castration on the thymus. He reported an increase in thymus weight, correlated with microscopic changes, in six rabbits which he castrated between the ages of 1 and 3 months and killed at 9 months. Similar results have been reported by Henderson ('04) for cattle, Goodall ('05) for guinea pigs, Masui and Tamura ('26) for mice, Wagenseil ('27) for men, Terni ('27) for birds, and Korenchevsky and Dennison ('34), as well as many others, for rats.

It has been shown in this paper that castration performed at various times between birth and 80 days resulted in definite increases in the weight of the thymus. The increase above normal was marked at 273 days and still present at 547 days if there were no complications from disease and infection. The time of onset of involution does not appear to be delayed in the castrate beyond the age of 80 days. The castrated animals merely have larger thymus glands. Most other investigators have restricted their observations to animals castrated before or at the time of puberty. Reports on post-puberal castrations are few in number (Hatai, '15; Lauson, Heller

and Sevringhaus, '37), especially those in which the animal was old enough at the time of castration to have an extensively involuted thymus.

Observations presented here show that the thymus is capable of returning to its former size and histological condition after extensive atrophy. One year after the thymus has decreased to about one-third of its puberal size, castration periods ranging from 20 to 60 days cause hyperplasia that entirely alters the gland's degenerated appearance. This investigation does not include a study of the possible origin of the new thymocytes. It was noted, however, that there is a tendency towards reconstitution of the dense cortical nodules in the center of the gland where the blood vessels are numerous. Although the thymus-altering effects of prepuberal castration largely disappear by 500 days of age, the normal involuted thymus at this time is capable of responding in a striking manner to short periods of castration. There appears to be in these experiments no sex difference in the extent of thymus hyperplasia resulting from castration.

Injections of various male and female hormone preparations caused involution of the thymus in both normal and castrated male and female rats. The decrease in thymus size was roughly proportional to the dose administered. In general a dose of hormone which was sufficiently large to stimulate the accessory reproductive organs was also large enough to cause a decrease in thymus weight.

It was found that the same thymus would increase in size after castration and then decrease following injections of sex hormones, or vice versa. These results resemble those of Jonson ('09) who showed that the thymus of the rabbit might be greatly reduced in weight through starvation and then brought back almost to normal after feeding was resumed.

Injections of gonad-stimulating hormones reduced the size of the thymus only in male and female rats which had their reproductive organs. They act, therefore, not directly on the thymus but only by invoking excessive sex hormone from the gonads.

Similar sex and gonadotropic hormone influences on the thymus have been reported by Golding and Ramirez ('28), Richter ('34), Evans and Simpson ('34) Korenchevsky, Dennison and Simpson ('35), Butcher and Persike ('38), Chiodi ('40) and others.

It is clear that the sex hormones have an influence on the thymus. This has been demonstrated by the effects of their removal and replacement. It is possible that at puberty the sex glands release hormones which bring about the involution of the thymus, principally by causing a gradual decrease in thymocytes in the gland. But other stimuli such as certain operative injuries, drugs, low temperature, and nervous excitement, produce effects on the thymus similar to those caused by the sex hormones (Selye, '36).

Many attempts have been made to determine whether the thymus is or is not an endocrine gland. Large numbers of experiments involving the feeding or injecting of thymus extracts (Rowntree, Clark and Hanson, '34; Asher, '36; Chiodi, '38 b; Nelson, '39; Smith and Jones, '40; Segaloff and Nelson, '40) and the implantation of thymus parenchyma (Einhorn and Rowntree, '38) have been reported. The results have been contradictory. Since much of the difficulty in such experiments lies in the actual nature of the thymus extracts, the opposite method of approach was attempted by the author, that is, thymectomy of rats.

A consideration of some of the literature dealing with the effects of thymectomy with special reference to the sex glands reveals that this approach has also resulted in contradictory findings.

Andersen ('32) reported that thymectomy of 21-day-old rats did not alter the onset of puberty as judged by the presence of motile spermatozoa in the tail of the epididymis. Other workers who have reported no change in testicular size nor influence upon the onset of spermatogenesis after removal of the thymus are Matti ('12) for the dog and rabbit, Halnan and Marshall ('14) for the guinea pig, Pap-

penheimer ('14) for the rat, Park and McClure ('19) for the dog, and Chiodi ('38 c) for the rat.

Testicular hypertrophy or hyperplasia have been reported by Paton ('05) after thymectomy of the guinea pig. Klose and Vogt ('10) obtained similar results in the dog and Adler ('14) in the frog. Paton and Adler believed that the thymus and sex glands act antagonistically before sexual maturity by exerting an inhibitory influence on each other. Soli ('07), however, reported a temporary arrest of testicular development following thymus removal in chickens, rabbits and guinea pigs. Lucien and Parisot ('10) confirmed these results in rabbits.

In the course of the present studies it was found that complete surgical removal of the thymus at 1 day of age had no effect on spermatogenesis of immature rats. These results are at variance with those reported by Shay, et al. ('39) after roentgen irradiation of the thymus and in accord with results recently reported by Reinhardt ('40) after surgical removal of the thymus. The author also found no effects on hormone output of testis, as judged by weights of seminal vesicles and their histological state.

This investigation has shown that the relationship between the thymus and the sex hormones in albino rats is not reciprocal. Removal of the gonads or injection of their hormones definitely affects the thymus. Surgical removal of the thymus, on the other hand, is without effect upon the gonads. This argues against the existence of an hormonic interrelationship.

The results of this paper may be interpreted to indicate that whatever the thymus function may be in albino rats it has no detectable influence on sex hormone secretion, germ cell production or body growth. Hence, in so far as the evidence goes the thymus, though showing response to castration and to sex hormone injection, does not appear to exert any influence on reproductive processes. From this study no evidence has emerged that the thymus is an organ of internal secretion.

SUMMARY

1. Normal growth and involution. The absolute weight of the thymus in normal male and female albino rats increases rapidly from birth to about 60 days of age. The gland then begins to show involution, decreasing rapidly in weight at first. The decrease in weight becomes slower between 200 and 300 days of age. The maximum weight at about 60 days of age is approximately 300 mg. The weight at 600 days of age is about 50 mg.

The maximum relative weight of the thymus of male and female rats is attained at about 25 days of age. This is followed by rapid involution at a time when body weight is increasing rapidly. After 150 days decreases in both absolute and relative weights proceed at the same rate. There is no clear-cut sex difference in the growth and involution of the thymus.

Involutionary changes are seen in the form and the histological appearance of the thymus. The two lobes of the involuted thymus retain their length but become slender and separated from each other. Histologically, the clear-cut differentiation between cortex and medulla seen during the height of thymus growth becomes indistinct in the involuted gland. Thymocytes disappear and the gland is invaded by connective tissue and fat.

Sexual maturity, from the standpoints of germ cell production, accessory sex organ growth, and actual reproductive capacity of both sexes is attained a short time before the thymus reaches its maximum development.

2. Effects of castration. When castration is performed prepuberally or at puberty the thymus responds by increasing in weight. This effect is apparent at the age of 273 days and suggested at 547 days. Although the thymus of a castrated rat is larger than that of the control at the age when involution of the normal thymus begins, involution in the castrate sets in at the usual time.

The extensively involuted thymus of a rat of advanced age is capable of great hyperplasia following relatively short

periods of castration. Gross and histological changes are such that the gland resembles the uninvoluted thymus. There appears to be no sex difference in the response of the thymus to castration.

3. Effects of hormone injections. The thymus regresses in weight following injections of androgens or estrogens into normal or castrated male or female animals or of gonadotropic hormones into animals in which the sex glands are intact. This response is roughly proportional to the dosage used. A quantity of hormone capable of stimulating the accessory reproductive organs is also effective in diminishing the size of the animal's thymus. The thymus may be first reduced in weight by sex hormones and then increased by castration. Conversely, the enlarged thymus of a castrate may be reduced in size by hormone administration. Histological changes similar to the early stages of age involution appear after injections of the hormones.

4. Effects of thymectomy. Thymectomy of the male rat at birth neither accelerates nor retards the progress of spermatogenesis as judged by the first appearance of sperm heads in the testis. Growth of the testis and its hormone output, judging from the appearance of the seminal vesicles, are not influenced.

Further observations on both male and female rats thymectomized between the ages of 10 and 16 days indicate that removal of the thymus has no effect in either sex on body weight, sexual maturity, appearance of hair, eruption of teeth, opening of eyes, breeding activity, or ability and rate of learning.

5. Conclusions. (1) The thymus is definitely sensitive to varying concentrations of sex hormones. (2) The reproductive system is not influenced by removal of the thymus. (3) Since the physiological relationship between the thymus and the reproductive system is not reciprocal, this study offers no evidence to support the claim that the thymus is an organ of internal secretion.

LITERATURE CITED

ADLER, L. 1914 Metamorphosestudien an Batrachierlarven. I. Exstirpation endokriner Drüsen. B. Exstirpation der Thymus. Arch. f. Entwcklngsmechn. d. Organ., vol. 40, pp. 1–17.

ANDERSEN, D. H. 1932 Studies on the physiology of reproduction. II. The effect of thymectomy on the age of puberty in the male rat. J. Physiol., vol. 74, pp. 207–211.

ASHER, L. 1936 Untersuchungen der hormonalen Funktionen der Thymus. Handb. d. biol. Arbeitsmeth., Abt. V, Teil 3 B [2], pp. 929–936.

BROWMAN, L. G. 1937 Light in its relation to activity and oestrous rhythms in the albino rat. J. Exper. Zool., vol. 75, pp. 375–388.

BUTCHER, E. O., AND E. C. PERSIKE, JR. 1938 The effect of antuitrin-S on the thymus of the young albino rat. Endocrinol., vol. 23, pp. 501–506.

CALZOLARI, A. 1898 Recherches expérimentales sur un rapport probable entre la fonction du thymus et celle des testicules. Arch. ital. de biol., vol. 30, pp. 71–77.

CHIODI, HUGO 1938 a Acción de la castración prepuberal sobre el timo y ganglios linfáticos de ratas albinas machos. Rev. Soc. argent. de biol., vol. 14, pp. 74–85.

———— 1938 b Acción de la injección del extracto de timo (Hanson) sobre el crecimiento y desarrollo de 5 generaciones sucesivas de ratas albinas. Rev. Soc. argent. de biol., vol. 14, pp. 326–330.

———— 1938 c Acción de la timectomía sobre el crecimiento y desarrollo de ratas albinas. Rev. Soc. argent. de biol., vol. 14, pp. 383–390.

———— 1940 The relationship between the thymus and the sexual organs. Endocrinol., vol. 26, pp. 107–116.

DONALDSON, H. H. 1924 The Rat. (Memoirs of The Wistar Institute of Anatomy and Biology, No. 6, Philadelphia.)

EINHORN, NATHAN H., AND LEONARD G. ROWNTREE 1938 The biological effects of thymus implantation in normal rats. Endocrinol., vol. 22, pp. 342–350.

EVANS, H. M., AND M. E. SIMPSON 1934 Reduction of the thymus by gonadotropic hormone. Anat. Rec., vol. 60, pp. 423–435.

GOLDING, G. T., AND F. T. RAMIREZ 1928 Ovarian and placental hormone effects in normal, immature, albino rats. Endocrinol., vol. 12, pp. 804–812.

GOODALL, ALEXANDER 1905 The post-natal changes in the thymus of guinea-pigs, and the effect of castration on thymus structure. J. Physiol., vol. 32, pp. 191–198.

HALNAN, E. T., AND F. H. A. MARSHALL 1914 On the relation between the thymus and the generative organs and the influence of these organs upon growth. Proc. Roy. Soc., London, s. B., vol. 88, pp. 68–89.

HAMMAR, J. A. 1906 Über Gewicht, Involution und Persistenz der Thymus im Postfötalleben des Menschen. Arch. f. Anat. u. Physiol., Anat. Abt., Suppl. Bd., pp. 91–182.

———— 1911 Zur Kenntnis der Elasmobranchier-Thymus. Zool. Jahbr., Abt. f. Anat., vol. 32, pp. 135–180.

HATAI, S. 1914 On the weight of the thymus gland of the albino rat (Mus norvegicus) according to age. Am. J. Anat., vol. 16, pp. 251–257.

———— 1915 The growth of organs in the albino rat as affected by gonadectomy. J. Exper. Zool., vol. 18, pp. 1–67.

HENDERSON, JAMES 1904 On the relationship of the thymus to the sexual organs. I. The influence of castration on the thymus. J. Physiol., vol. 31, pp. 222–229.

JACKSON, C. M. 1913 Postnatal growth and variability of the body and of the various organs in the albino rat. Am. J. Anat., vol. 15, pp. 1–68.

JONSON, ARVID 1909 Studien über die Thymusinvolution. Die akzidentelle Involution bei Hunger. Arch. f. mikrosk. Anat. u. Entwcklngsch., vol. 73, pp. 390–443.

KLOSE, H., AND H. VOGT 1910 Klinik und Biologie der Thymusdrüse. Mit besonderer Berücksichtigung ihrer Beziehungen zu Knochen- und Nervensystem. Beitr. z. klin. Chir., vol. 69, pp. 1–200.

KORENCHEVSKY, V., AND M. DENNISON 1934 The manifold effects of castration in male rats. J. Path. & Bact., vol. 38, pp. 231–246.

KORENCHEVSKY, V., M. DENNISON AND S. L. SIMPSON 1935 The effects of water soluble preparations of androsterone and androsterone-diol on castrated rats. Biochem. J., vol. 29, part 2, pp. 2131–2142.

LAUSON, HENRY, CARL G. HELLER AND ELMER L. SEVRINGHAUS 1937 The effect of graded doses of estrin upon the pituitary, adrenal, and thymus weights of mature ovariectomized rats. Endocrinol., vol. 21, pp. 735–740.

LUCIEN, M., AND J. PARISOT 1910 Contribution à l'étude des fonctions du thymus. Son influence sur la croissance, le développement du squelette et l'évolution des organs. Arch. de méd. expér et d'Anat. path., vol. 22, pp. 98–137.

MARINE, D., O. T. MANLEY AND E. J. BAUMANN 1924 The influence of thyroidectomy, gonadectomy, suprarenalectomy, and splenectomy on the thymus gland of rabbits. J. Exper. Med., vol. 40, pp. 429–443.

MASUI, K., AND Y. TAMURA 1926 The effect of gonadectomy on the weight of the kidney, thymus, and spleen of mice. Brit. J. Exper. Biol., vol. 3, pp. 207–223.

MATTI, H. 1912 Untersuchungen über die Wirkung experimenteller Auschaltung der Thymusdrüse. Mitt. a. d. Grenzgeb. d. Med. u. Chir., vol. 24, pp. 665–821.

MOORE, CARL R., AND DOROTHY PRICE 1932 Gonad hormone functions, and the reciprocal influence between gonads and hypophysis with its bearing on the problem of sex hormone antagonism. Am. J. Anat., vol. 50, pp. 13–71.

———— 1938 Some effects of testosterone and testosterone-propionate in the rat. Anat. Rec., vol. 71, pp. 59–78.

NELSON, WARREN O. 1939 "The relation of the thymus and pineal glands to genital function," Sex and Internal Secretion, 2nd ed. (The Williams and Wilkins Company, Baltimore.)

PAPPENHEIMER, ALWIN 1914 The effects of early extirpation of the thymus in albino rats. J. Exper. Med., vol. 19, pp. 319–338.

PARK, E. A., AND R. D. MCCLURE 1919 The results of thymus extirpation in the dog with a review of the experimental literature on thymus extirpation. Am. J. Dis. Child., vol. 18, pp. 317–521.

PATON, D. N. 1905 The relationship of the thymus to the sexual organs. II. The influence of removal of the thymus on the growth of the sexual organs. J. Physiol., vol. 32, pp. 28–32.

PLAGGE, JAMES C. 1939 Effects of gonadectomy on the involuted thymus of albino rats. Anat. Rec., vol. 75 (suppl.), p. 76 (abstract).

———— 1940 a Effect of thymectomy at birth on spermatogenesis in the albino rat. Proc. Soc. Exper. Biol. & Med., vol. 44, pp. 57–60.

———— 1940 b The thymus gland in relation to sex hormones and reproductive processes in the albino rat. (Doctorate thesis. The University of Chicago Library.)

REINHARDT, WILLIAM O. 1940 Effect of thymectomy in immature rats. Proc. Soc. Exper. Biol. & Med., vol. 43, pp. 732–733.

RICHTER, C. P. 1934 Pregnancy urine given by mouth to gonadectomized rats: its effect on spontaneous activity and on the reproductive tract. Am. J. Physiol., vol. 110, pp. 499–512.

ROWNTREE, L. G., J. H. CLARK AND A. M. HANSON 1934 The biologic effects of thymus extract (Hanson). Accruing acceleration in growth and development in successive generations of rats under continuous treatment with thymus extract. J. A. M. A., vol. 103, pp. 1425–1430.

SEGALOFF, ALBERT, AND W. O. NELSON 1940 Growth of vitamin deficient rats treated with thymocrescin. Endocrinol., vol. 26, pp. 860–862.

SELYE, HANS 1936 Thymus and adrenals in the response of the organism to injuries and intoxications. Brit. J. Exper. Path., vol. 17, pp. 234–248.

SHAY, HARRY, JACOB GERSHON-COHEN, SAMUEL S. FELS, DAVID R. MERANZE AND THEODORE MERANZE 1939 The thymus: Studies of some changes in the gonads and pituitary following its destruction by roentgen irradiation. J. A. M. A., vol. 112, pp. 290–292.

SMITH, GEORGE VAN S., AND E. ELIZABETH JONES 1940 Attempt to modify growth, development and tumor incidence in mice with thymus gland extracts. Proc. Soc. Exper. Biol. & Med., vol. 43, pp. 157–160.

SÖDERLUND, G., AND A. BACKMAN 1909 Studien über die Thymusinvolution. Die Altersveränderungen der Thymusdrüse beim Kaninchen. Arch. f. mikrosk. Anat. u. Entwcklngsch., vol. 73, pp. 699–725.

SOLI, U. 1907 Comment se comportent les testicules chez les animaux privés du thymus. Arch. ital. de biol., vol. 47, pp. 115–122.

TERNI, TULLIO 1927 Sulle modificazioni istolotiche prodotta nel timo dalla castrazione e dall' età. Monitore zool. ital., vol. 38, pp. 300–308.

WAGENSEIL, F. 1927 Beiträge zur Kenntnis der Kastrationsfolgen und des Eunuchoïdismus beim Mann. Ztschr. f. Morphol. u. Anthropol., vol. 26, pp. 264–304.

PRESS OF
THE WISTAR INSTITUTE
OF ANATOMY AND BIOLOGY
PHILADELPHIA